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Analyses of Avocado (*Persea americana*) Nectar Properties and their Perception by Honey bees (*Apis mellifera*)

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Abstract Honey bees are important avocado pollinators. However, due to the low attractiveness of flowers, pollination is often inadequate. Previous work has revealed that avocado honey is relatively unattractive to honey bees when compared with honey from competing flowers. We characterized avocado honey and nectar with respect to their odor, color, and composition of sugars, phenolic compounds, and minerals. Furthermore, we tested how honey bees perceive these parameters, using the proboscis extension response bioassay and preference experiments with free-flying bees. Naïve bees were indifferent to odors of avocado and citrus flowers and honey. Experienced bees, which were collected in the field during the blooming season, responded preferentially to odor of citrus flowers. The unique sugar composition of avocado nectar, which contains almost exclusively sucrose and a low concentration of the rare carbohydrate perseitol, and the dark brown color of avocado honey, had no negative effects on its attractiveness to the bees. Phenolic compounds extracted from avocado honey were attractive to bees and adding them to a solution of sucrose increased its attractiveness. Compared with citrus nectar and nonavocado honey, avocado nectar and honey were rich in a wide range of minerals, including potassium, phosphorus, magnesium, sulfur, iron, and copper. Potassium and phosphorus, the two major minerals, both had a repellent effect on the bees. Possible explanations for the presence of repellent components in avocado nectar are discussed.

Keywords Honey · Citrus · Repellence · Pollination · Minerals · Phenolic compounds · Potassium · Phosphorus · Proboscis extension response · *Apis mellifera* · *Persea americana* · Nectar

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Introduction

Avocado (*Persea americana*: Lauraceae) originated in the neotropics where it is naturally pollinated by a wide range of insects, mainly stingless bees and social wasps (Ish-Am et al., 1999; Can-Alonzo et al., 2005). It is an important crop in many tropical and subtropical regions around the world (Knight, 2002). Its open and rather small (1 cm diam) flowers can also be visited by honey bees, which are its main pollinators in agricultural landscapes (Gazit and Degani, 2002). However, even when colonies of honey bees are placed inside the orchards, avocado flowers often suffer from insufficient pollination activity, resulting in low fruit yields (Vithanage, 1990; Ish-Am, 1998; Ish-Am and Eisikowitch, 1998; Gazit and Degani, 2002).

The attractiveness of nectar to pollinators is probably most affected by its taste (Adler, 2000), but may also be affected by odor (Raguso, 2004) and color (Thorp et al., 1975). The taste of nectar is dominated by a high sugar concentration. Several studies have attempted to define whether honey bees prefer nectars that are rich in sucrose or hexose. Wykes (1952) showed that honey bees prefer a sugar ratio of 1:1:1 (sucrose/fructose/glucose) over a pure sucrose solution. Avocado nectar contains almost exclusively sucrose and a low concentration of the unique seven-carbon sugar alcohol, perseitol (Ish-Am, 1994; Liu et al., 1995; Dvash et al., 2002; Dag et al., 2003). Indeed, the high ratio of sucrose in avocado nectar has been suggested as the cause for the low attractiveness of avocado flowers to honey bees (Ish-Am, 1994). A preference for hexoses over sucrose is also suggested by physiological considerations, because sucrose has to be broken down before it can be utilized (Harborne, 1993). Other studies, however, have found that honey bees are indifferent to the ratio of sugars (Southwick et al., 1981), or even prefer a high sucrose concentration (Bachman and Waller, 1977; Hagler et al., 1990). The sensitivity of honey bees to perseitol has never been evaluated.

Other components of nectar, including minerals, phenolic compounds, and amino acids, may make a cardinal contribution to its attractiveness to honey bees. Minerals such as potassium (Waller et al., 1972) and sodium chloride (von Frisch, 1950) deter honey bees. Phenolic compounds affect the taste of nectar even at very low concentrations (Baker, 1977), and in some cases they have been suspected of repelling honey bees (Hagler and Buchmann, 1993; Adler, 2000). Several nectar amino acids have also been shown to affect preference (Kim and Smith, 2000; Gardener and Gillman, 2002; Carter et al., 2006). Ish-Am (1994) found that glycine and histidine are the dominant amino acids in some avocado cultivars; at their naturally occurring concentrations, however, these amino acids do not repel honey bees (Inouye and Waller, 1984) and may even attract them (Kim and Smith, 2000). Avocado nectar is poor in proline, which is attractive to honey bees (Carter et al., 2006), but so is citrus (cv. "Valencia") nectar, and yet it is highly attractive to bees (Ish-Am, 1994). Hence, it does not appear that amino acid composition can explain the low attractiveness of avocado nectar.

Odors guide bees toward flowers and may affect their attractiveness (von Frisch, 1967). The volatile components of nectar, including phenolic compounds, form particular odor bouquets (Anklam, 1998). Some of these compounds are more attractive to honey bees than others (Jay, 1986; Henning et al., 1992; Winston and Slessor, 1993).

Colors also affect the attractiveness of flowers (von Frisch, 1967; Giurfa et al., 1995), and nectar may contribute to their visual display (Thorp et al., 1975). Color differences are especially salient when comparing honeys, which are derived from nectar, and can be used in choice experiments for testing the influence of nectar components on bees' preferences

(Afik et al., 2006). Nectar and honey colors are produced by dissolved light-absorbing compounds. Whereas sugar solution is transparent, dark honeys such as avocado honey have relatively high concentrations of minerals (Petrov, 1970; Terrab and Heredia, 2004; Dag et al., 2006). Other compounds, including flavonoids (Anklam, 1998) and organic acids (Mato et al., 2003), may also affect honey color, in either the visible or ultraviolet spectra, which are visible to bees (Hagler and Buchmann, 1993).

Previous work has revealed that avocado honey is relatively unattractive to honey bees when compared with honey from competing flowers (Afik et al., 2006). These results suggest that some attributes of avocado nectar are responsible for the relatively low attractiveness of avocado flowers. Here, we studied the influence of odor, color, sugar composition, phenolic compounds, and minerals on the selection of sugar source by honey bees.

Methods and Materials

Mineral Composition

We measured mineral composition in avocado honey, nonavocado honey, avocado (cv. "Ettinger") nectar, and citrus (cv. "Shamouti") nectar. The honey was extracted from colonies placed in avocado orchards during the blooming season. Its avocado origin was confirmed by sugar analysis, perseitol constituting 2.5% of the total sugars (Dvash et al., 2002). The nonavocado honey was extracted from colonies placed in citrus orchards during the citrus blooming season; it contained no perseitol. Nectar samples were collected from flowers by hand using microcapillary tubes. Flowers were covered with paper bags on the evening before collection to prevent nectar consumption by insects.

Nitric acid (5 ml of 65%, w/w) was added to the two honey and two nectar samples. Samples were prepared for analysis by microwave-assisted digestion, using an MLS 1200 mega microwave digestion unit [Milestone Sorisole (BG) Italy] at 500 W for 10 min. Liquid residues were taken up in deionized water to a final volume of 25 ml. Concentrations of the different elements were determined simultaneously by inductively coupled plasma–atomic emission spectrometry (ICP-AES), according to EPA 6010B (1996), using two ICP-AES systems, models "Spectroflame" and "Spectroflame Modula E" from Spectro (Kleve, Germany).

Behavioral Bioassay—Proboscis Extension Response

Odor

We employed the proboscis extension response (PER) bioassay to test the responses of harnessed bees to the odors of avocado and citrus flowers and honey. In this bioassay, subjects extend their proboscis in response to an odor associated with an appetitive reinforcement (Bitterman et al., 1983). The experiment was conducted in April 2003, in Rehovot, Israel. We tested bees from two colonies. One hive was located in the field, between citrus and avocado orchards, during their simultaneous blooming period. Thus, the foragers from this colony may have visited citrus and avocado flowers before being tested. A second hive was introduced into a 12 × 6 × 3 m enclosure (15 mesh) before the blooming period to avoid preconditioning. This colony was fed sugar solution and pollen patties during the experimental period.

Each morning, 30 bees from one colony were caught in glass vials as they flew out of the hive. The vials were placed on ice for 1 or 2 min until the bees were motionless, and then the bees were strapped into a sectioned hollow plastic tube (6 mm diam), with a 3-mm-wide strip of duct tape that wrapped around the tube and (dorsal) thorax of the bee (Shafir et al., 1999). When they awoke, bees were fed 5 μl of a 30% (w/w) sucrose solution. Typically, only a few bees did not feed, and they were removed from the experiment. Each day, 24 bees were chosen and allowed to adapt to the harness for 1 hr. We tested a total of 142 bees from the colony in the orchard and 215 bees from the colony in the enclosure.

During the experiment, odor was delivered to each bee for 3 sec. An air pump delivered air through valves controlled by a computer, and a Tygon tubing connected to a 50-ml plastic syringe filled with fresh flowers or a 130-ml glass vial filled with 30 ml honey solution, diluted to a total sugar concentration of 60% (w/w). The vial was placed in a warm water bath (40°C; Abramson and Boyd, 2001). Tubing from the odor source delivered odors to within 2 cm of the bee.

Each bee experienced five different odors with an intertrial interval of 10 min. The first two presented were of avocado flowers ("Ettinger" or "Fuerte" cultivars) and citrus flowers ("Shamouti"). The order of odor presentation was alternated every day. Because these were unrewarded trials, after the first two trials the bees were fed 2 μl of 30% sucrose solution to avoid starvation. The subsequent three trials included odors of avocado honey, nonavocado honey, and an air control. The order of the two honey sources was alternated every day, but the air control was always last. An extension of the proboscis in response to a particular odor was considered a positive response and the proportion of bees responding to each odor was calculated.

Sugars

The sensitivity of honey bees to increasing concentrations of various sugar solutions was tested in February and July of 2002, respectively, in two sets of PER experiments (Page et al., 1998). The first experiment tested the sensitivity of bees to different sugars ($N = 150$ bees, 37–38 bees for each sugar). The second tested the bees' sensitivity to different sugar mixtures ($N = 275$ bees, 68–69 bees for each mixture). The bees were caught and harnessed as described for the odor experiment. Each trial with a sugar concentration was preceded by a trial with distilled water, which served as a baseline to which the effect of the sugar component of the solution was compared. The intertrial interval was 4 min.

The experiment was begun by touching the right antenna of each bee with a cotton ball soaked in distilled water. The PER to the touch was recorded, and in the next trial the same antenna was touched again, this time with a cotton ball soaked in a sugar solution. We rotated between antennae so that the next two trials (water and increasing sugar solution) were to the left antenna, and so forth. The total sugar concentrations of the examined solutions were as follows: 0.1%, 0.3%, 1%, 3%, 10%, and 30% w/w (g solute/g solution).

On each day of the experiment, the tested bees were separated randomly into four different groups, which were tested for their response to four different sugar solutions. In the first experiment, each solution contained only a single sugar: sucrose, glucose, fructose, or perseitol, with an added concentration of 4.5% (w/w), the highest concentration that could be reached using perseitol. In the second experiment, four sugar mixtures were used: (1) "avocado nectar": 95% sucrose and 5% perseitol, to simulate the sugar composition of avocado nectar (Ish-Am, 1994); (2) "perseitol-enriched": 90% sucrose and 10% perseitol; (3) "citrus nectar": 50% fructose, 30% glucose, and 20% sucrose, to simulate the sugar composition of citrus nectar (Ish-Am, 1994); (4) sucrose solution, used as a reference.

Behavioral Bioassay—Free-Flying Bees

General Procedure

The effects of honey color, and phenolics and mineral composition, were studied with a cafeteria-style choice paradigm, in which free-flying honey bees could choose from three available feeders. Five-frame nucleus hives were kept in screened enclosures. For the honey color and phenolics experiments, colonies were kept in a 12 × 6 × 3 m (15 mesh) enclosure. Only one colony was tested at a time, and the entrances to the other colonies were closed the evening before each test. The mineral composition experiment was conducted in 5 × 2.5 × 2 m (20 mesh) enclosures, each housing one colony. The bees had *ad libitum* access to a water source and were provided with a pollen patty once a week.

Three different honey solutions were prepared each day by diluting honey or sucrose with distilled water to reach 60% (w/w) total dissolved solids. The concentration was measured by a hand refractometer (REF 114, brix units, 28–62 ATC). Although the tested honeys contain mainly glucose and fructose (Dag et al., 2006), their refractive index is similar to that of sucrose (Kearns and Inouye, 1993). The three different solutions used in the experiments were avocado honey, nonavocado honey, and sucrose.

The solutions were presented to the bees in 200-ml bird feeders. The three feeders were placed in a circle, 15 cm apart, on a carousel that rotated at a velocity of 2 rpm, to prevent a potential location bias. The volume consumed from each feeder was measured every 20 min. The experiment ended when 130 ml were consumed from one of the feeders, or after 4 hr had elapsed. The volume of solution consumed from each feeder was measured at the end of the experiment. The solution from which bees consumed the highest volume was set to represent 100% consumption, and the consumed volumes from the rest of the feeders were compared with this highest volume. The results are presented as relative percentage consumption from the different solutions.

Color

Avocado honey is characterized by its dark brown color (Terrab and Heredia, 2004; Dag et al., 2006). To test whether the bees' preference for nonavocado over avocado honey is influenced by color (Afik et al., 2006), we tested 10 colonies in a preference experiment in which the feeders were covered with an opaque green plastic cover. A narrow opening (marked in yellow) was left at the bottom of the cover, allowing bees to reach the feeder inside. The choice was between avocado honey, nonavocado honey, and sucrose solution.

Phenolic Compounds in Honey

To test the effect of phenolic compounds in avocado honey on preference, an experiment was conducted in which bees had a choice between dilute avocado honey, sucrose solution with a phenolic concentrate produced from avocado honey, and sucrose solution with ethanol (the solvent). The phenolic concentrate was made by dissolving 600 g of avocado honey in 1.2 l distilled water; 200 ml of ethyl acetate was mixed with the honey solution and then separated from the solution using a funnel separator. This process was repeated five times. All ethyl acetate fractions were pooled, and the solvent was evaporated under reduced pressure on ice (Singleton et al., 1999). The residue was dissolved in 2 ml ethanol, because such a low concentration of ethanol is known to have only a minor effect on honey bees (Abramson et al., 2000).

Each feeder of avocado honey solution contained 200 g of honey (200 ml of 60% diluted honey solution; Weast, 1988). Therefore, the 2 ml phenolic concentrate that was extracted from 600 g of honey was divided into three portions of 0.67 ml, which sufficed for three replicates of the preference test, with three different colonies. Similarly, 0.67 ml of ethanol was added to each feeder of sucrose solution.

Potassium

To test the effect of potassium concentration in honey on preference, an experiment was conducted in which bees had a choice between avocado honey, nonavocado honey, and nonavocado honey enriched with potassium. Potassium concentrations were 3768 and 325 mg/kg in the avocado and nonavocado honeys, respectively (Table 1). We, therefore, dissolved 3443 mg potassium in 1 kg nonavocado honey solution to reach a concentration equivalent to that of the avocado honey solution. Four different potassium salts were used: potassium chloride (KCl), potassium hydrogen phosphate (K_2HPO_4), potassium D-gluconate ($C_6H_{11}KO_7$), and tri-potassium citrate ($C_6H_5K_3O_7$). The amounts of potassium salts added were calculated from their molecular weights to reach the desired concentration of potassium. Eight colonies were tested for each of the four salts. Each day, all the colonies were tested for the same potassium salt, with 1 or 2 d between tests. Because the addition of potassium salts did not noticeably change the color or odor of the honey, the feeders were placed on colored cardboards (yellow, blue, or purple) to allow the bees to visually discriminate between the solutions. The colors were rotated between colonies and between days of the experiment to avoid potential color bias.

Statistical Analyses

Differences in the responses of harnessed bees to odors were tested by χ^2 test. The sugar sensitivity experiment was analyzed by two-way ANOVA, including the honey source as a nominal factor, the log of the sugar concentration as a continuous factor, and the proportion of discriminating bees at each sugar concentration as the dependent variable. A discriminating bee was defined as a bee that extended its proboscis in the trial with sugar solution but not in the preceding trial with water. This provided a conservative measure of the sensitivity to the sugar itself. Differences in the preferences of free-flying bees between

Table 1 Concentrations (mg/kg) of detected minerals in avocado and nonavocado honey and nectar

Mineral	Avocado honey	Nonavocado honey	Avocado nectar	Citrus nectar
K	3768.3	324.8	3946.2	184.7
P	651.5	47.0	511.2	18.5
Mg	204.6	18.5	188.3	<5
S	188.3	27.7	170.4	<5
Ca	82.7	75.8	<150	<150
Na	58.9	79.1	53.8	18.5
Si	18.0	7.0	43.9	29.5
Zn	10.9	1.5	<30	<30
B	9.9	7.2	10.8	4.2
Fe	9.3	2.7	13.5	<5
Cu	3.2	0.1	3.1	<0.5
Pb	1.2	2.9	<1	<1

honey sources were tested by one-way ANOVA. Differences between pairs of treatments within the same experiment were tested by Tukey–Kramer test. The arcsin square root transformation was employed on the percentage honey consumption data before analysis (Sokal and Rohlf, 1995). Statistical analyses were performed using JMP 5.0.1 software (SAS Institute, Inc.).

Results

Odor

Approximately half of the bees from the enclosure responded spontaneously to floral odors, and there was no difference between the response rates to avocado and citrus flowers ($\chi^2_{1,428} = 3.03$, $P > 0.05$; Fig. 1a). The response of the orchard bees to the odor of avocado flowers was similar to that of the bees from the enclosure ($\chi^2_{1,355} = 0.27$, $P > 0.05$), but a significantly higher proportion responded to citrus flowers than to avocado flowers ($\chi^2_{1,282} = 16.8$, $P < 0.001$).

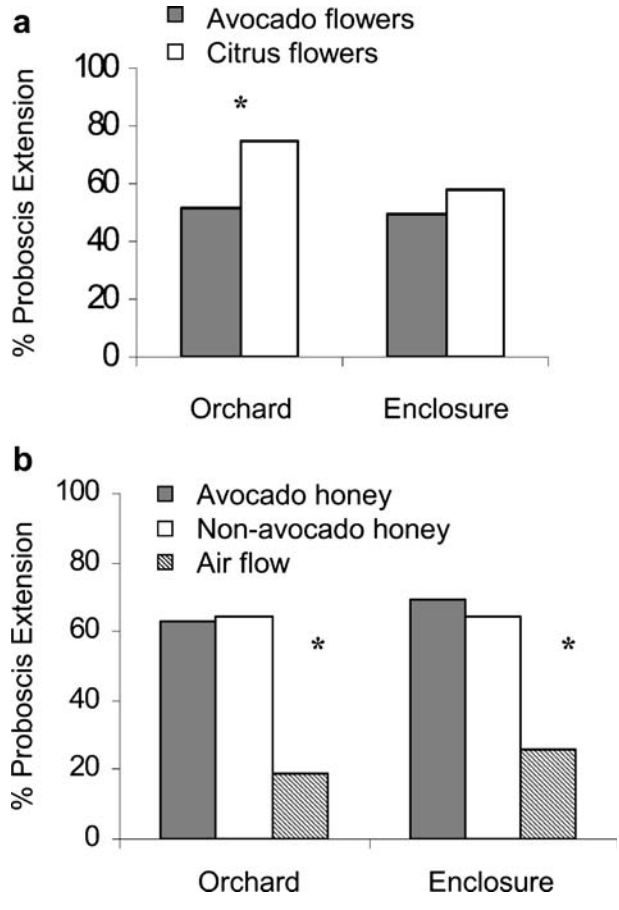

Fig. 1 The percentage of bees that responded spontaneously, by proboscis extension, to different odors: (a) floral odors and (b) honey odors. Bees from two colonies were tested. One hive was placed in an orchard (N = 142 bees) and the second in an enclosure (N = 215 bees). * indicates significant differences between treatments with $P < 0.001$ (χ^2 test)

The spontaneous response rate of bees to honey odors was 65%, and it was not affected by honey source ($\chi^2_{1,712} = 0.40$, $P > 0.05$; Fig. 1b) or location ($\chi^2_{1,712} = 0.74$, $P > 0.05$). The spontaneous response to the air flow was lower than the responses to either honey odor ($\chi^2_{2,1067} = 176.7$, $P < 0.001$).

Sugars

Visual inspection shows that bees started detecting sugars only at concentrations >1% (Fig. 2). Therefore, we analyzed the effect of sugar type and concentration for concentrations of ≥1%. In the first experiment, which measured the sensitivity of bees to different sugars, bees were most sensitive to the sucrose solution (Fig. 2a). Both sugar type ($F_{3,10} = 14.7$, $P = 0.001$) and concentration ($F_{1,10} = 31.2$, $P < 0.001$) affected discrimination, but the interaction between them was not significant ($F_{3,10} = 1.99$, $P > 0.05$). Nevertheless, separate linear regressions for each sugar showed that the percentage of discriminating bees increased with increased sucrose ($F_{1,3} = 139.5$, $P = 0.001$) and glucose ($F_{1,3} = 41.5$, $P = 0.008$) concentration, while no concentration effect was evident for fructose ($F_{1,3} = 6.21$, $P > 0.05$) or perseitol ($F_{1,1} = 0.23$, $P > 0.05$).

In the second experiment, which measured the bees' sensitivity to four sugar mixtures, bees responded similarly to all sugar mixtures ($F_{3,8} = 1.77$, $P > 0.05$; Fig. 2b). Sensitivity

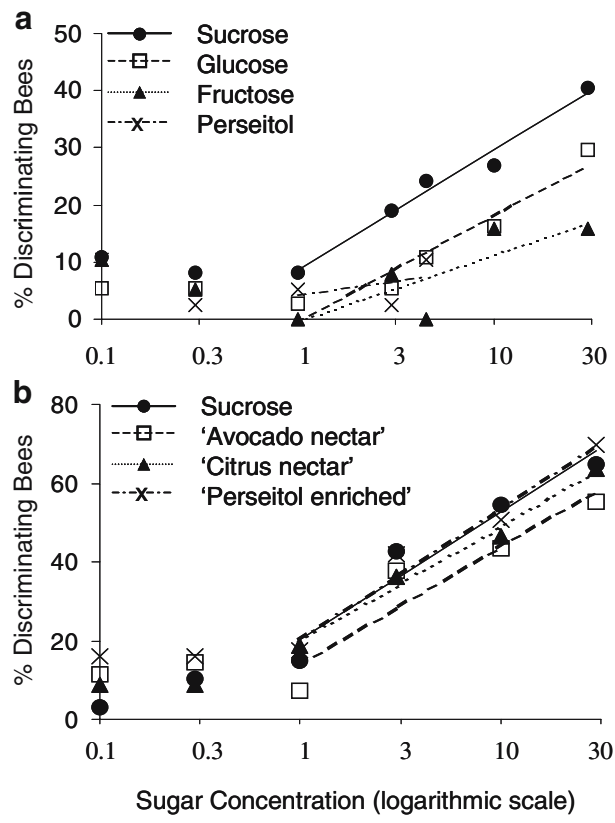

Fig. 2 The percentage of bees that discriminated between having their antennae touched with cotton balls soaked with distilled water and sugar solution according to their proboscis extension response. (a) The discrimination percent in response to different sugars at increasing concentrations ($N = 150$ bees). (b) The discrimination percent in response to different sugar mixtures at increasing concentrations ($N = 275$ bees). Lines are linear best fits

increased with concentration ($F_{1,8}$ = 127.0, $P < 0.001$) in a similar fashion for all sugar mixtures (sugar mixture × concentration: $F_{3,8}$ = 0.12, $P > 0.05$).

Color

Repellence by avocado honey was observed even when opaque feeders were used, and bees could not discriminate between the solutions by their color ($F_{2,27}$ = 44.9, $P < 0.001$; Fig. 3a). Consumption of avocado honey was lower than that of nonavocado honey and sucrose solution (Tukey's test, $P < 0.05$). No significant differences were found in final consumption between nonavocado honey and sucrose solution (Tukey's test, $P > 0.05$).

The consumption rate of the solutions was measured every 20 min. Figure 3b shows the average rate of the first, middle, and last 20-min increments of the experiment for every colony. Consumption rate was affected by the solution source ($F_{2,81}$ = 18.8, $P < 0.001$), by the time point in the experiment ($F_{2,81}$ = 13.8, $P < 0.001$), and by the interaction between

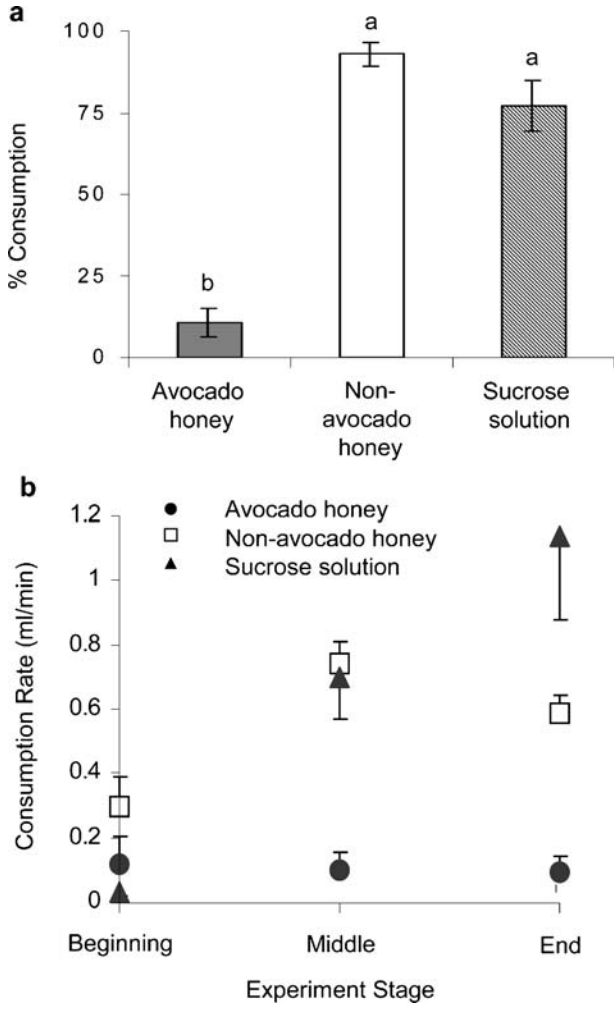

Fig. 3 (a) Bees' mean (±S.E.) relative consumption of different sugar solutions presented in covered feeders (N = 10 colonies). Relative consumption represents the ratio between the amount consumed from each solution and the amount consumed from the solution with the highest consumption, for each colony tested. Different letters indicate significant differences between treatments with $P < 0.05$ (Tukey's test). (b) Consumption rate (ml/min ± S.E.) as the experiment progressed. Beginning—The rate during the first 20 min of the experiment. Middle—The rate during 20 min in the middle of the experiment. End—The rate during the last 20 min of the experiment

them ($F_{4,81}$ = 7.46, P < 0.001). Consumption rate of the nonavocado honey was highest after the first 20 min and increased until the middle of the experiment. Consumption rate of the sucrose solution showed a different pattern. Sucrose was consumed the most slowly at the beginning, but the rate increased consistently, and it was consumed at the highest rate toward the end of the experiment.

Phenolic Compounds

Consumption of the sucrose solution enriched with phenolics from avocado honey was higher than that of the sucrose solution or of the avocado honey ($F_{2,6}$ = 30.6, P = 0.001; Fig. 4), with no significant difference between the latter two (Tukey's test, P > 0.05), probably due to the strong attraction to the enriched sucrose solution.

Mineral Composition

Twelve different minerals were found in avocado honey at concentrations higher than 1 mg/kg (Table 1). The concentrations of 10 minerals out of the 12 were higher in the avocado honey than in the nonavocado honey, in some cases by an order of magnitude. Among the minerals that were detected in the honey samples, nine were also detected in avocado nectar, and only five in citrus nectar, all of them with higher concentrations in avocado than in the citrus nectar. Potassium was the most dominant mineral in all samples, with a concentration in avocado honey and nectar that was >10-fold that in nonavocado honey and citrus nectar.

Potassium

The consumption of nonavocado honey enriched with potassium salts was intermediate between those of nonavocado honey and avocado honey (Fig. 5). Three salts significantly reduced consumption relative to nonavocado honey, but were still preferred to avocado honey (KCl: $F_{2,21}$ = 42.4, P < 0.001; K-gluconate: $F_{2,21}$ = 101.3, P < 0.001; K_3-citrate: $F_{2,21}$ = 234.5, P < 0.001). Only K_2-phosphate reduced consumption to the level of avocado honey ($F_{2,21}$ = 85.5, P < 0.001).

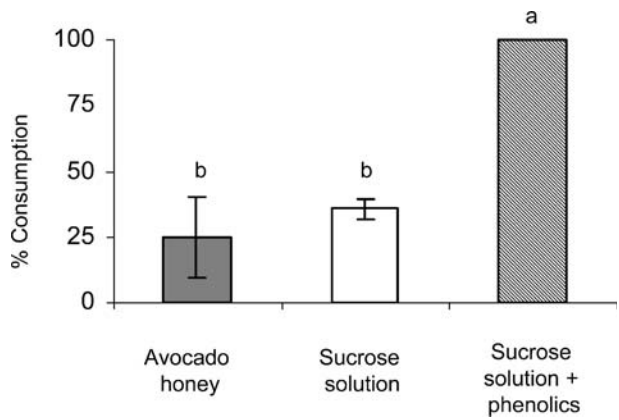

Fig. 4 Bees' mean (±S.E.) relative consumption of solutions containing avocado honey, sucrose solution, and sucrose solution enriched with phenolics extracted from avocado honey. Different letters indicate significant differences between treatments with P < 0.05 (N = 3 colonies; Tukey's test)

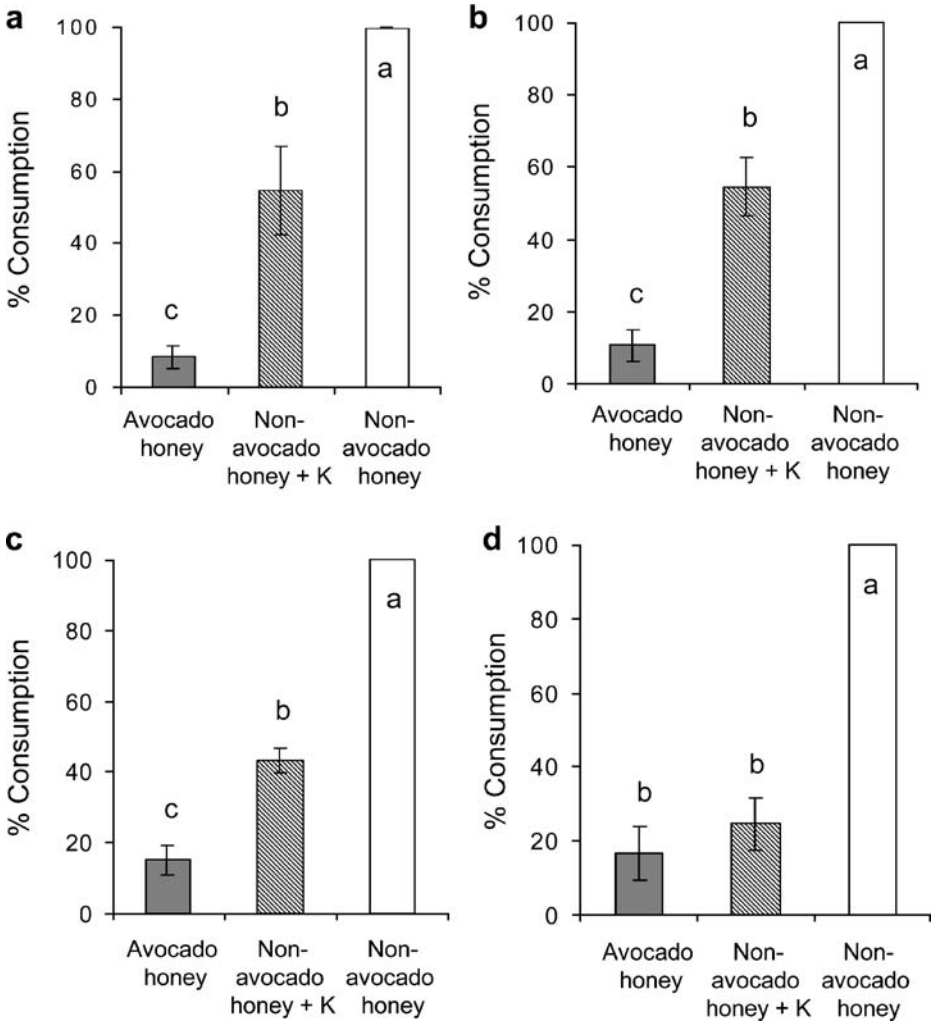

Fig. 5 Bees' mean (±S.E.) relative consumption of solutions containing avocado honey, nonavocado honey, and nonavocado honey enriched with different potassium salts: (a) KCl, (b) K-gluconate, (c) K_3-citrate, and (d) K_2-phosphate. Different letters indicate significant differences between treatments with $P < 0.05$ (N = 8 colonies for each potassium salt; Tukey's test)

Discussion

The dissolved solids in nectar consist mainly of carbohydrates (Luttge, 1977), but a wide variety of minor components may define its nature (Adler, 2000). This complicates the identification of individual taste and odor compounds that may affect its attractiveness to pollinators. Here, we separated various constituents of nectar and tested their effects on the preferences of honey bees. The response of honey bees to floral and honey odors evaluated the importance of volatile compounds. The odor of citrus flowers was more attractive to experienced bees than that of avocado flowers. These foragers were collected from a colony located among blooming citrus and avocado trees. Because honey bees tend to prefer citrus

over avocado (Vithanage, 1990; Ish-Am and Eisikowitch, 1998; Gazit and Degani, 2002), it is likely that we collected more bees that were foraging on citrus than on avocado. The higher response to the odor of citrus flowers probably reflected the bees' foraging experience in the field. The indifference of naïve bees to the floral odors supports the notion that odors act mainly as signals that bees learn to associate with their respective floral rewards, and do not themselves affect choice behavior greatly. This view was also supported by the indifference of bees to honey odors, while they clearly preferred nonavocado honey in taste assays. Taste, rather than odor, probably affected the choice of honey, because honey bees find it difficult to discriminate between honey odors (Bonod et al., 2003).

The response of bees to different sugar solutions indicated that the sugar composition of avocado nectar cannot explain its low attractiveness. Their sensitivity to sucrose was found to be higher than their sensitivity to glucose or fructose, separately, thus supporting the findings of Wykes (1952), Waller (1972), and Bachman and Waller (1977). Sugar mixtures, however, are perceived differently relative to pure sucrose (Wykes, 1952; Waller, 1972; Bachman and Waller, 1977). The response to a hexose-rich mixture, "citrus nectar" in the present experiments, was similar to that of sucrose. Therefore, it appears that high sucrose content in avocado nectar does not diminish its attractiveness, in contrast to previous assumptions (Ish-Am, 1994). Bees did not respond to perseitol solution, and their response to sucrose solution containing perseitol was similar to their response to pure sucrose solution. Perseitol, which was suspected to deter honey bees from avocado (Ish-Am, 1994; Can-Alonzo et al., 2005), seems to have no effect on preference. Thus, differences in sugar composition between avocado and citrus nectar cannot account for the bees' stronger preference for citrus flowers. Moreover, during the process of honey ripening, sucrose is inverted into glucose and fructose. As a result, the sugar compositions of avocado and nonavocado honeys are similar (Dag et al., 2006). Nevertheless, higher consumption of nonavocado honey in the color and potassium experiments indicated that preference is determined by a component other than the dominant sugars.

Honey color also could not explain the bees' preferences. Bees were repelled by the avocado honey even when feeders were covered. Comparison of the consumption rate as the experiment progressed revealed a potential role of odor in establishing preference. The honey solutions would be easy to locate due to their aromas, whereas sucrose solution is odorless. Bees visiting the nonavocado honey feeder would further mark it with Nasanov pheromone (Winston, 1987), and bees visiting the avocado honey feeder would abandon it. Thus, a rapid preference for the nonavocado honey feeder would develop (Fig. 3b). Eventually, bees finding the sucrose solution would start marking it with pheromone, and by the end of the experiment, it did indeed attract the greatest number of bees, revealing that it is even more attractive than nonavocado honey.

Phenolic compounds have been suspected of being repellent to bees (Baker, 1977; Rhoades and Bergdahl, 1981; Adler, 2000), but actual repellence has seldom been found. Hagler and Buchmann (1993) tested two phenolic compounds and found that they were repellent at high concentrations, but at low concentrations they did not deter bees and even increased attractiveness. They also found repellence to phenolic-rich nectars and honeys from three different botanical sources. One of them was almond honey, which was later found to be repellent due to amygdalin (London-Shafir et al., 2003). Another source was salt cedar, which contains high potassium concentrations (Waller et al., 1972). Our results indicated that phenolics increase the attractiveness of sucrose solution to honey bees, probably by adding odor to the solution, making it easier to locate. A similar increase in

visitation rate to phenolic-rich nectar was found for *Apis cerana* (Liu et al., 2004). Hence, no support was found for a repellent effect of phenolics on honey bees.

Little is known about the effect of nectar minerals on honey bee foraging behavior (Nicolson and W.-Worswick, 1990). A repellent effect of sodium chloride in sucrose solution was demonstrated by von Frisch (1950), who showed that a 0.015 M salt solution deterred honey bees, although a 0.0075 M solution no longer deterred them. The sodium component in the latter solution was 173 ppm. We found lower sodium concentrations than that in all honeys and nectars tested, and they were within a similar range in all samples. Hence, sodium does not seem to be responsible for deterring bees from avocado. Waller et al. (1972) found a repellent effect of potassium in onion nectar, at potassium concentrations similar to those found in the current study for avocado nectar. Our results indicate that, for three out of four potassium salts tested, dissolving 3500 ppm potassium in nonavocado honey to equalize its concentration with that of avocado honey decreases consumption by half. This indicates that potassium is a major cause for the low attractiveness of avocado honey, but not the only one. Cations such as potassium are usually accompanied by anions, although selective secretion to nectar is also possible (Luttge, 1977). In avocado nectar, potassium may be coupled with perseitol (Ishizu et al., 2001). This is supported by the strong correlation between perseitol and potassium in avocado honey (Dag et al., 2006). The most dominant anion found in avocado honey was phosphate, but its concentration was too low to equalize the potassium concentration. Adding K_2-phosphate to nonavocado honey reduced consumption to a level similar to that of avocado honey. Thus, high mineral concentrations, mainly of potassium and phosphate, seem to be the main cause for the low attractiveness of avocado flowers. The effect of other minerals, such as magnesium, sulfur, and copper, whose concentrations differ between avocado and nonavocado honeys, still remains to be tested. Potassium concentration in avocado nectar may often be higher than that measured in this study and, therefore, high enough to repel honey bees from avocado flowers. Potassium concentration in avocado nectar is high, but not unique (Hiebert and Calder, 1983; Waller et al., 1972). The effect of nectar minerals, potassium in particular, on the foraging behavior of pollinators may be widespread.

The reason for the presence of repellent components in nectar is not clear, but several possible roles have been suggested (Rhoades and Bergdahl, 1981; Adler, 2000). To answer this question for avocado, we have to compare the honey bees' response with avocado nectar, these insects not being its natural pollinators, with the response of avocado's natural pollinators. These natural pollinators may not be as strongly repelled by the nectar. For example, various hummingbird species have been found to differ in their response to mineral-rich nectar (Bouchard et al., 2000). Another approach would be to test whether potassium concentration in the nectar correlates with its abundance in the soil. It is possible that in avocado's natural habitat, the infertile soils of the neotropical rainforest (Wolstenholme, 2002), potassium concentration in the nectar is lower than in cultivated plots, and does not repel pollinators. It would also be interesting to study the influence of the agricultural practice of intensive fertilization of avocado (Lahav and Whiley, 2002) on the pollination effectiveness of bees.

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